(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 648 503 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **94114195.4**

(51) Int. Cl.[6]: **A61K 47/48**

(22) Date of filing: **09.09.94**

(30) Priority: **22.09.93 EP 93115234**

(43) Date of publication of application:
19.04.95 Bulletin 95/16

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

(71) Applicant: **BEHRINGWERKE Aktiengesellschaft**
Postfach 1140
D-35001 Marburg (DE)
Applicant: **LABORATOIRES HOECHST S.A.**
Tour Roussel Nobel, Cédex 3
F-92080 Paris La Défense (FR)

(72) Inventor: **Bosslet, Klaus, Dr.**
An der Haustatt 64
D-35037 Marburg (DE)
Inventor: **Czech, Jörg, Dr.**
Kreutzacker 2a
D-35041 Marburg (DE)
Inventor: **Hoffmann, Dieter, Dr.**
Feuerdornweg 12
D-35041 Marburg-Elnhausen (DE)
Inventor: **Tillequin, François**
70 rue de l'Amiral Mouches
F-75013 Paris (FR)
Inventor: **Florent, Jean-Claude**
23 rue des Causus
F-91940 Les Ulis (FR)
Inventor: **Azoulay, Michel**
16 rue du Regard
F-75006 Paris (FR)
Inventor: **Monneret, Claude**
9 avenue Lamoricière
F-75012 Paris (FR)
Inventor: **Jacquesy, Jean-Claude**
46 rue du Planty
F-86360 Buxerolles (FR)
Inventor: **Gesson, Jean-Pierre**
"La Germonière", Montassie
F-86360 Chansseneuil du Poitou (FR)
Inventor: **Koch, Michel**
116 Elysées II
F-78170 La Celle Saint Cloud (FR)

(74) Representative: **Lauppe, Hans Friedrich**
Behringwerke AG
Abt. Patente & Verträge,
Postfach 1140
D-35001 Marburg (DE)

(54) Pro-prodrugs, their production and use.

(57) Substrate-spacer-prodrug compounds (pro-prodrugs) suited for site specific delivery of drugs, a process of preparing them and their use are described.

EP 0 648 503 A1

The invention refers to the field of substrate-spacer-prodrug compounds (pro-prodrugs) suited for site specific delivery of drugs, a process of preparing them and their use.

A prodrug may be defined as a chemical which is non-toxic and pharmacodynamically inert, but which can be transformed in vivo to a pharmacologically active drug.

Prodrug transformation can be obtained in vivo using site specifically located enzymes (WO 92/19639), systemically available enzymes (Etoposide phosphate, Ref.: 10th International Congress of Chemotherapy, June 27 - July 2, 1993, Abstract Nr. 692), endogenous intracelluar lysosomal proteolytic enzymes (Duncan R., Anti-Cancer Drugs 3, 175-210, 1992) or sequential action of two endogenous "tumor site specific" enzymes (WO 93/08688). This enzyme mediated transformation is most effective with respect to pharmacological efficacy, - if the **prodrug is highly detoxified** compared to the drug -, can be **selectively activated at the target site**, and has an **extended plasma half-life.** Detoxification is optimally obtained, if the hydrophilicity of the prodrug is significantly higher than that of the drug combined with a suitable plasma stability of the prodrug. Prodrug transformation is optimally obtained, if the prodrug can be target site specifically cleaved with high activation rate (Vmax/Km). In addition, plasma half life of prodrugs can be extended by linking to hydrophilic high molecular weight copolymers (Duncan, R., AntiCancer Drugs 3, 175-210, 1992).

The prodrugs reported in the literature do not fulfill the criteria for optimal site specific prodrug activation.

Now, new pro-prodrugs with exceptional pharmacological efficacy could be synthesized harbouring all the favourable parameters mentioned above. These pro-prodrugs need activation by at least two different enzymes, one of them located accessible only at the target site, the other one located systemically or in a different compartement/site.

One of the enzymes can be an endogenous enzyme located in the plasma (i.e. esterases, preferentially phosphatases, etc.), the second enzyme could be an endogenous intracellular, preferentially a lysosomal enzyme (i.e. proteases, glycosidases, preferentially glucuronidases, etc.) accessible only at the target site or a targeted enzyme. Targeting preferentially can be obtained using an appropriate fusion protein (EP-A 0501215) or an antibody-enzyme conjugate (WO 88/07378).

Furthermore, pro-prodrug activation can be performed by independent or sequential action of the two enzymes. Independent action means that the pro-prodrugs are substrates for both enzymes; sequential action means that the pro-prodrugs are only substrates for one enzyme and after conversion to prodrugs are substrates for the second enzyme. An independent action of both enzymes is preferable for the efficient release of the drug at the target site. Especially, the combined activity of locally accessible endogenous lysosomal or targeted glycosidases and systemically available esterases/phosphatases results in very efficient target site specific activation.

The pro-prodrugs according to the invention consist of the following common building blocks:

$(prodrug)_x$-S I

wherein

S means a substrate moiety to which one or more (x = 1 or an integer) prodrug moieties can be bound and wherein the prodrug-S bond or bonds can be cleaved by one enzyme and where for optimal cleavage S can be linked to the prodrug moiety by a spacer which can be self-immolative, whereby a self-immolative spacer is defined as a moiety which is bound through two bonds to two molecules and which eliminates itself from the second molecule if the bond to the first molecule is cleaved and

wherein

prodrug means a moiety which can be cleaved by another enzyme to generate a pharmacologically active substance and

wherein the activating enzymes are at least two different human enzymes one of these located accessible only at the target site of the body.

Preferred S moieties are hydrophilic moieties such as mono- or oligophosphate, sulfate, dicarboxylate or polymeric moieties such as polyglutamate, polysialic acid, polyethylen glycol or N-(2-hydroxypropyl)-methacrylamide copolymers and

preferred prodrugs are compounds of formula II:

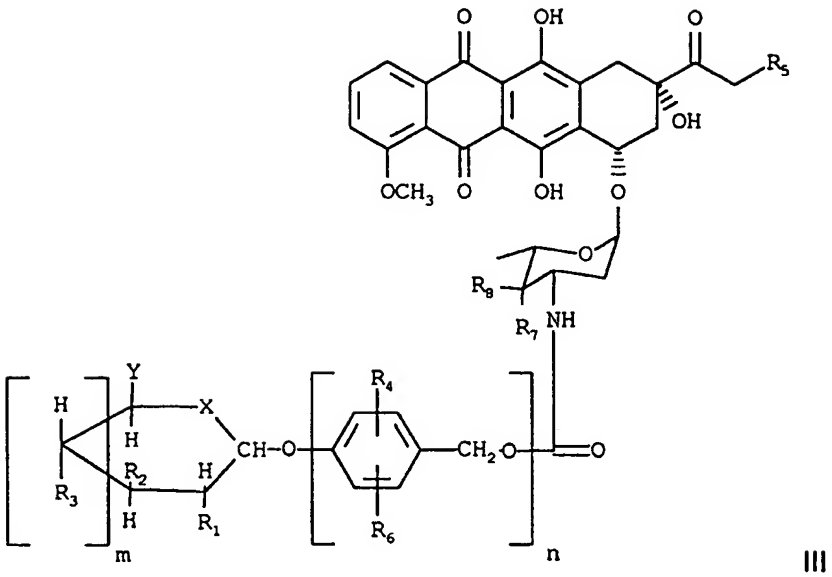
glycosyl-spacer-drug II

wherein

glycosyl means a mono-, oligo- or polysaccharid or derivative thereof spacer means a moiety as described above and

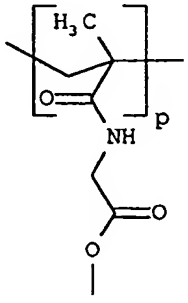
drug means a pharmacologically active substance.

Especially preferred are compounds according to formula III

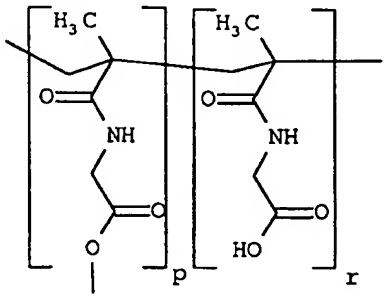


III

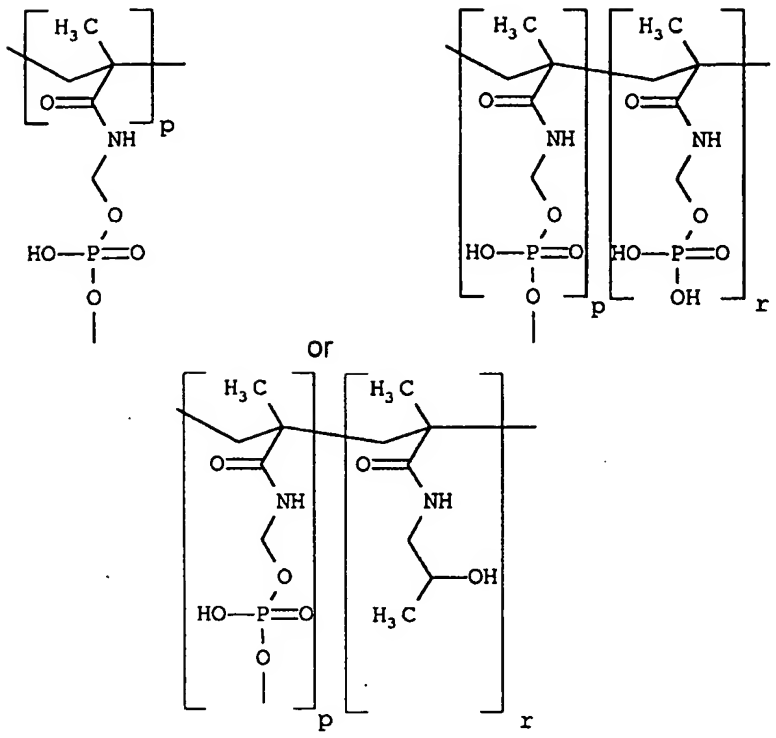
wherein
one of $R_{1,2,3,4,5,7,8}$ may be mono- or oligophosphate, sulfate, an acyl moiety such as $-OCO(CH_2)_o-COOH$ where o is 0 to 100, preferentially 0 to 30, a polymeric residue



or



where p and r are 0 to 100, preferentially 1 to 30,
a polyglutamat where p is 0 to 100, preferentially 1 to 30,
a phosphodiester



where p and r are 0 to 100, preferentially 1-30
or a phosphodiester substituted with polyethylene glycol or with polysialic acid
and the other R's are independently of each other H or OH,
wherein R_6 may be an electron withdrawing residue, preferentially a nitro-, fluoro- or chloro-residue,
wherein n is 0 or 1,
wherein X is O, NH or S,
wherein Y may be COOH, PO_3H_2, $-CH_2-COOH$, $-CH_2-PO_3H_2$, CHOH-COOH or CHOH-PO_3H_2,
wherein m is 0 or 1.

Preferred are compounds, which need activation by at least two different enzymes, one of them located accessible only at the target site, the other one located systemically or in a different compartement/site, compounds, wherein the activating enzymes are esterases, preferentially phosphatases, combined with glycosidases, preferentially β-glucuronidases.

Enzyme in this application may also mean catalytic antibody.

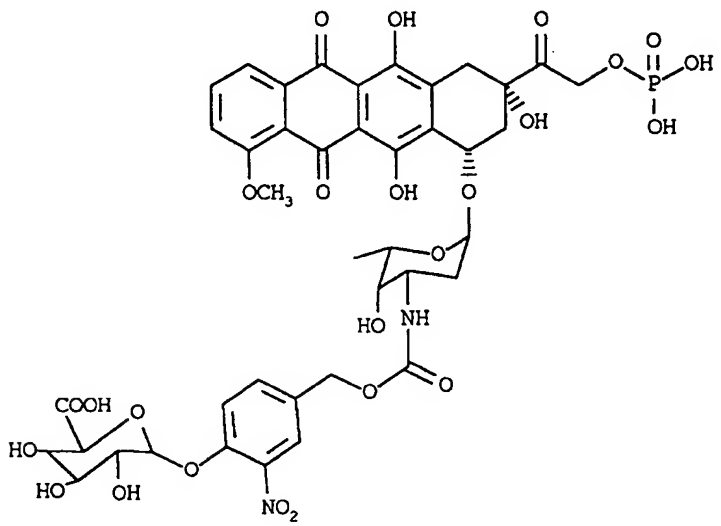
Preferred embodiments of the invention are depicted in the following. If not described, the compounds can be prepared by prior art methods, for example by activation of the 14-C position of N-[3-nitro-4-(methyl (β-D-glucopyranosyl) uronat)-benzyloxycarbonyl] daunorubicin (described as compound 64b in WO 92/19639) by halogenation according to F. Arcamone (German Patent P 1917874) and further reaction of this intermediate with the desired ligand for example a mono- or dibasic carboxylic acid as described by M. Israel (J. Med. Chem., 1986, 29, 1273-1276). Hydrolysis of the methyl (β-D-glucopyranosyl) uronat moiety to yield the free glucopyranosyluronat can be done according to WO 92/19639 either before or after coupling of the desired ligand.

14-O-Sulfate can be prepared analogously to the phosphate.

Derivatives of N-(4-β-glucuronyl-3-nitrobenzyloxycarbonyl)-doxorubicin-14-O-phosphate, substituted at the phosphate moiety, can be obtained according to J.-B. Ducep (German Patent P 2943438) by reaction of a N-[3-nitro-4-(methyl (β-D-glucopyranosyl) uronat)-benzyloxycarbonyl] daunorubicin-14-halide with the corresponding substituted phosphate. Substances modified at the glucuronic acid or the sugar moiety of the anthracyclin can be obtained by the synthesis according to WO 92/19639, starting with the desired modified glucuronic acid moiety or the sugar modified anthracycline with appropriate blocking groups if necessary.

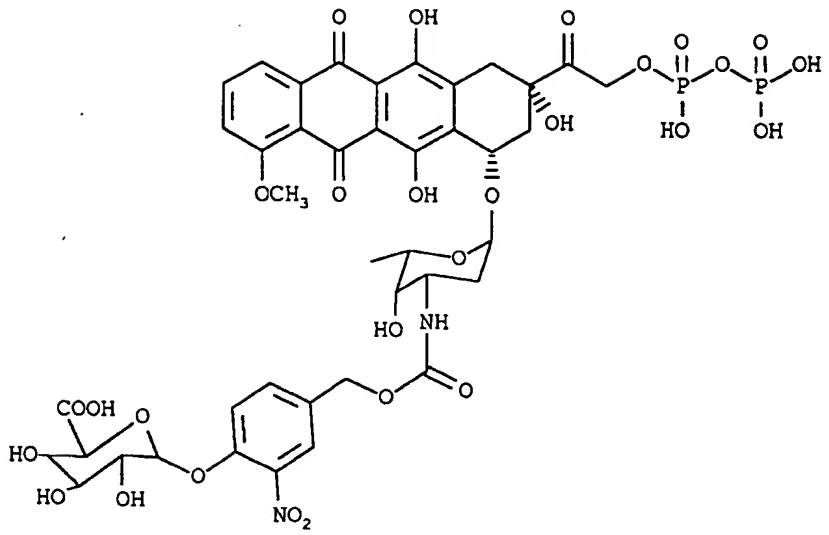
Example 1:

N-(4-β-glucuronyl-3-nitrobenzyloxycarbonyl)-doxorubicin-14-O-phosphate

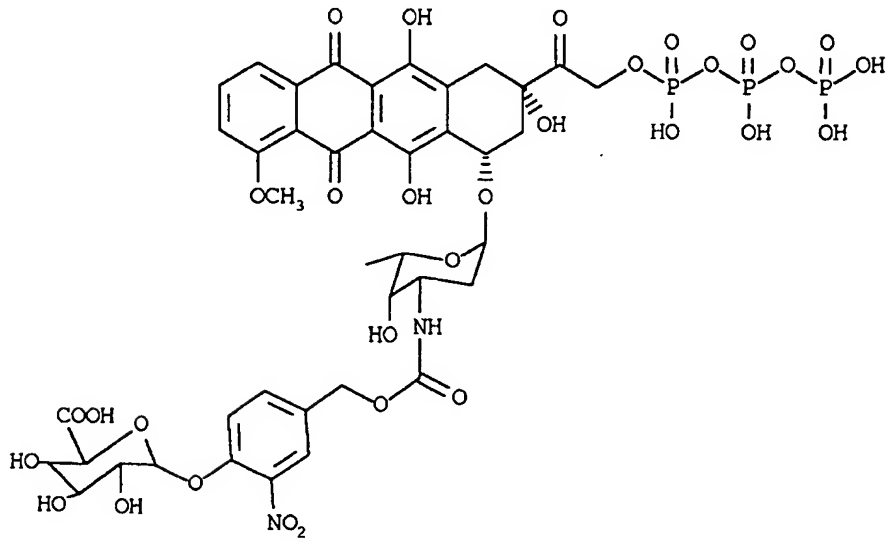


To a solution of N-[3-nitro-4-(methyl (β-D-glucopyranosyl) uronat)-benzyloxycarbonyl] doxorubicin (described as compound 70b in WO 92/19639) (100 mg, 0.11 mmol) in dry acetonitrile (5 ml) was added 1.1 eq. of phosphoryl chlorid (10 µl in 1 ml of acetonitril), in presence of 4 eq. of N,N'-diisopropylethylamine (76 µl in 1 ml of MeCN). The reaction mixture was stirred overnight, and then evaporated (T < 40 °C) under reduced pressure to give a syrup. The later was dissolved in a mixture of THF (5 ml) and H_2O (3 ml) and cooled at 0 °C then, a 2 N aqueous solution of NaOH was added (6 eq., 0.3 ml) and the reaction mixture stirred for 3 hrs. The mixture was carefully acidified until pH = 8 before evaporation to dryness. The crude final product was purified on a C18 silicagel column. The later was washed with H_2O and the product was eluted with aq. MeCN.

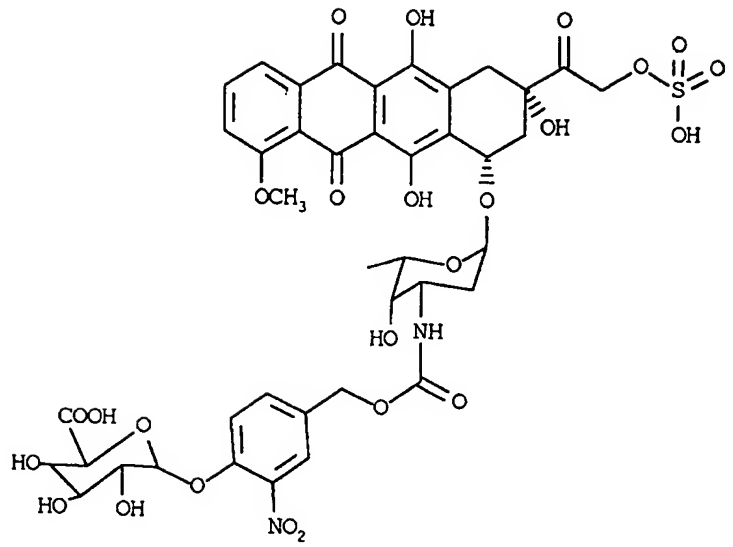
Example 2:



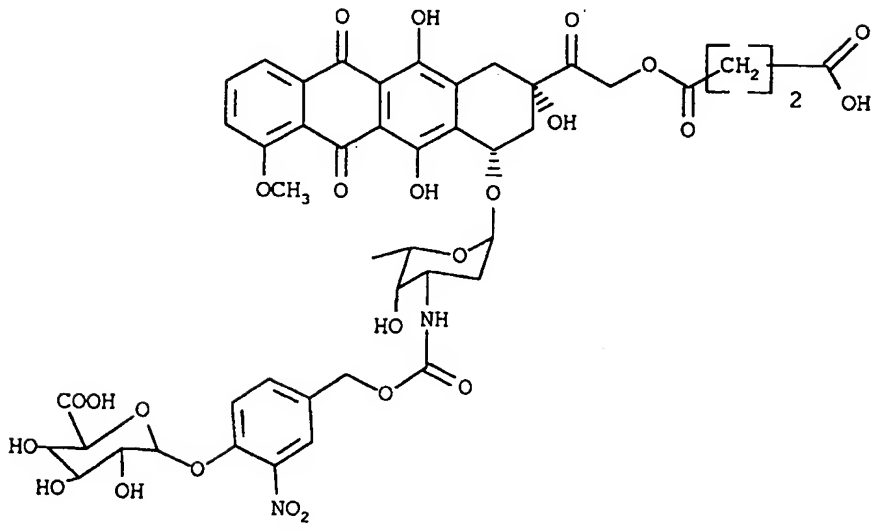
Example 3:



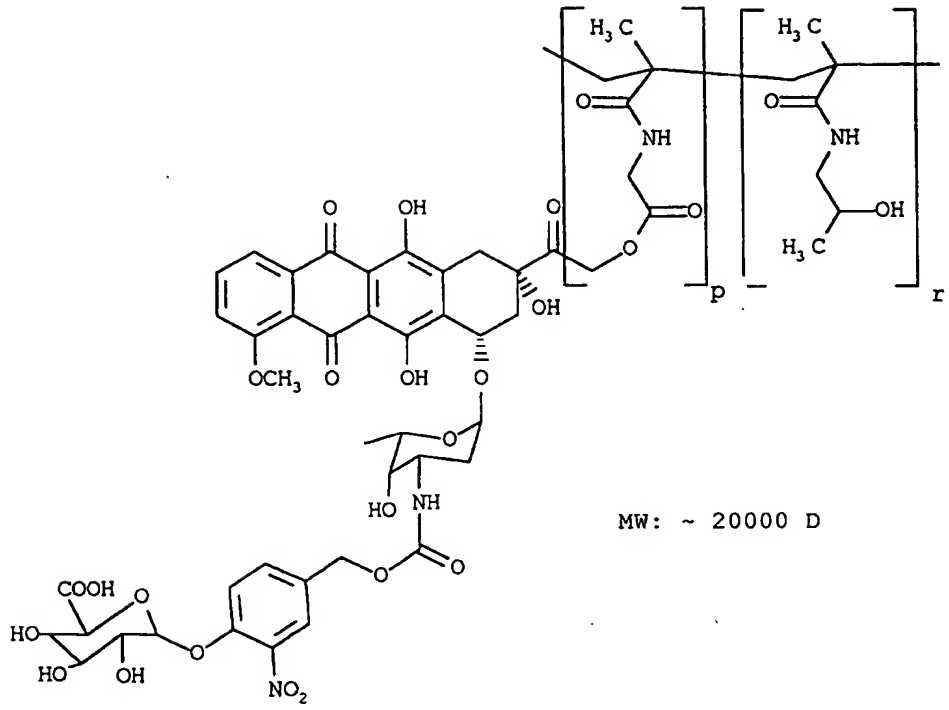
Example 4:



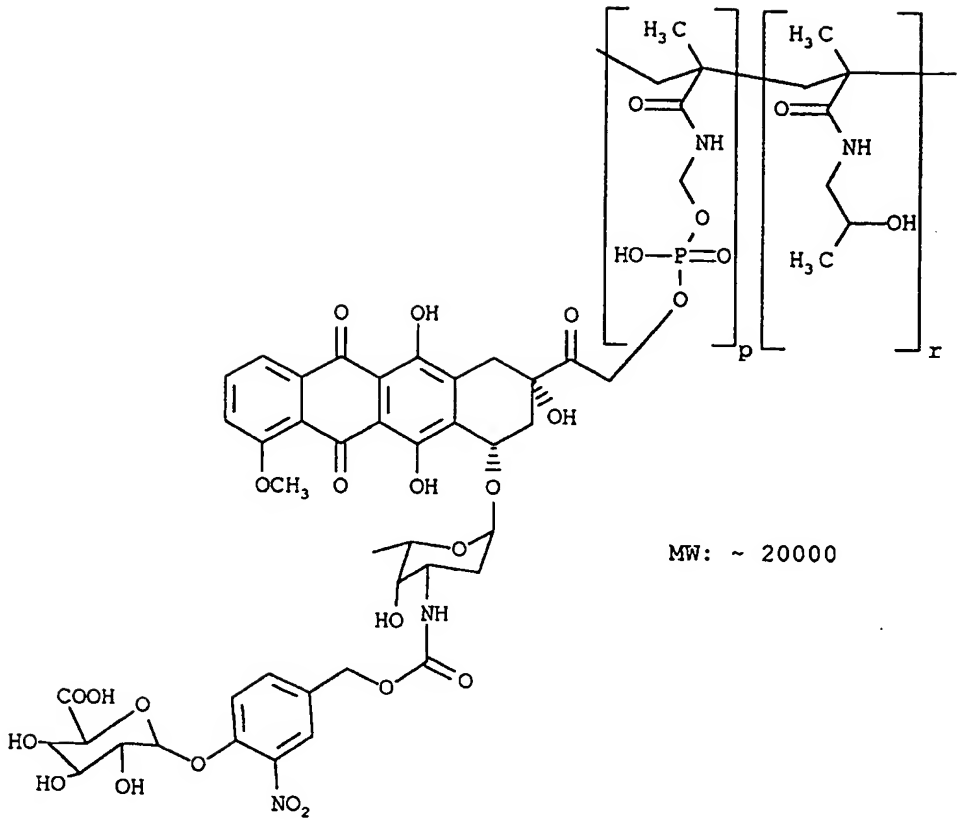
Example 5:



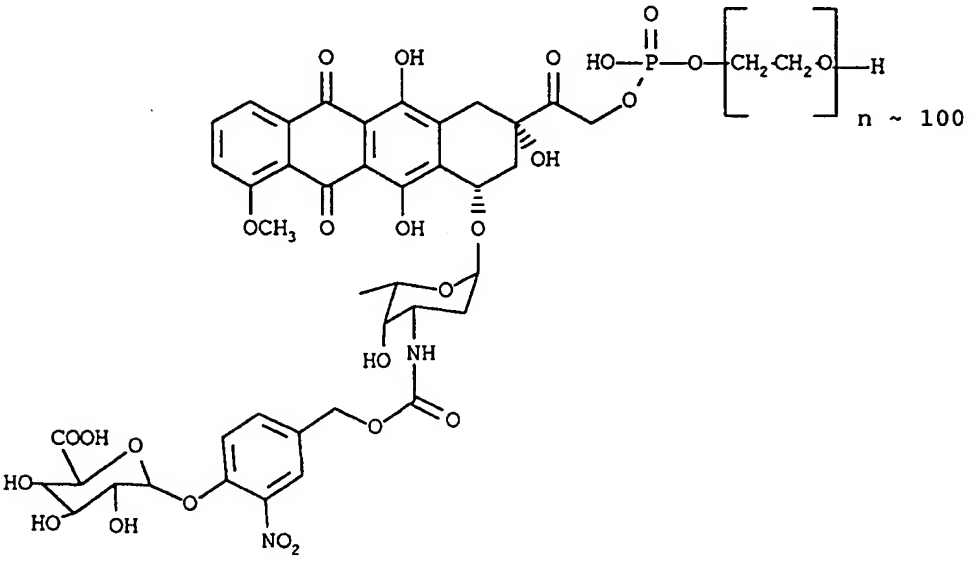
Example 6:



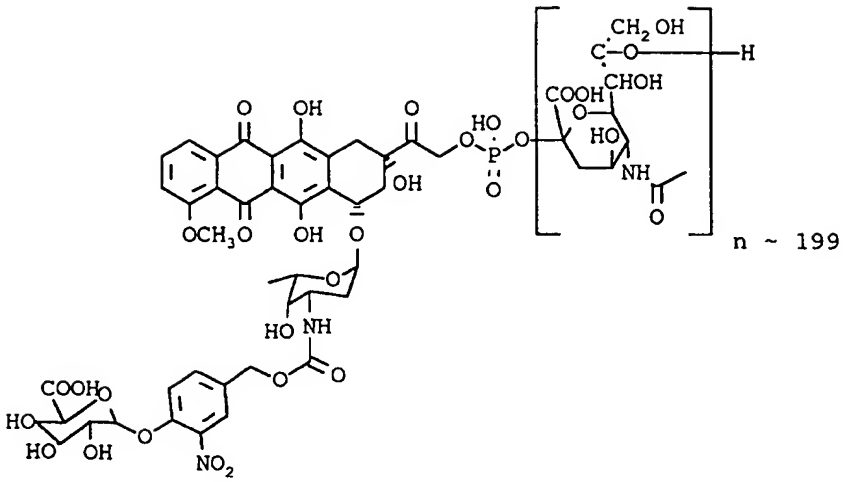
Example 7:



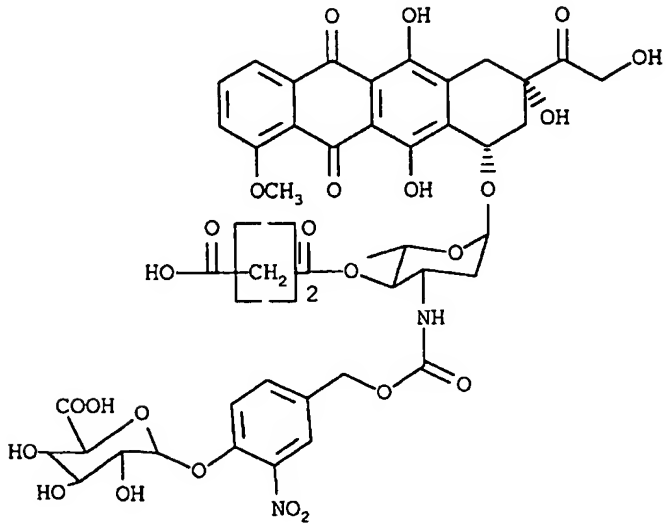
Example 8:



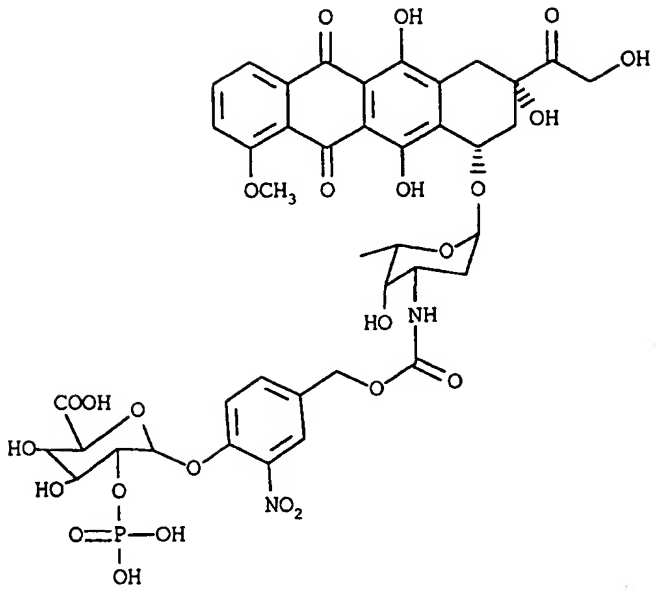
Example 9:



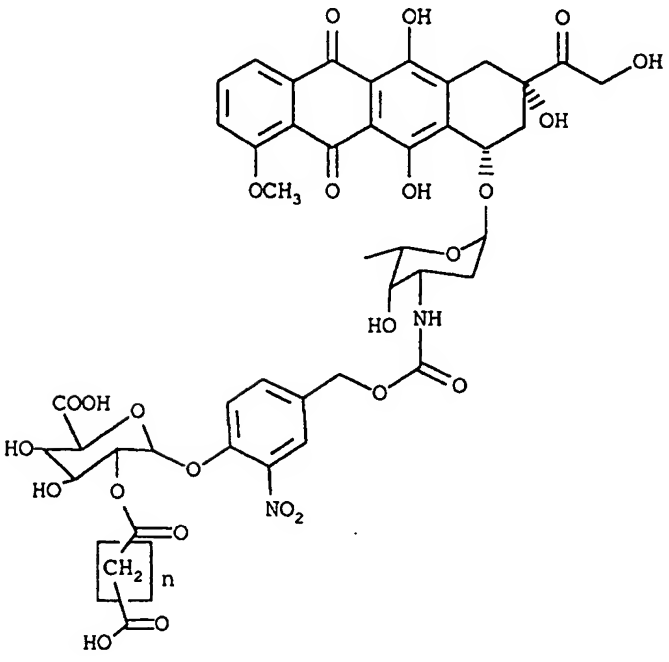
Example 10:



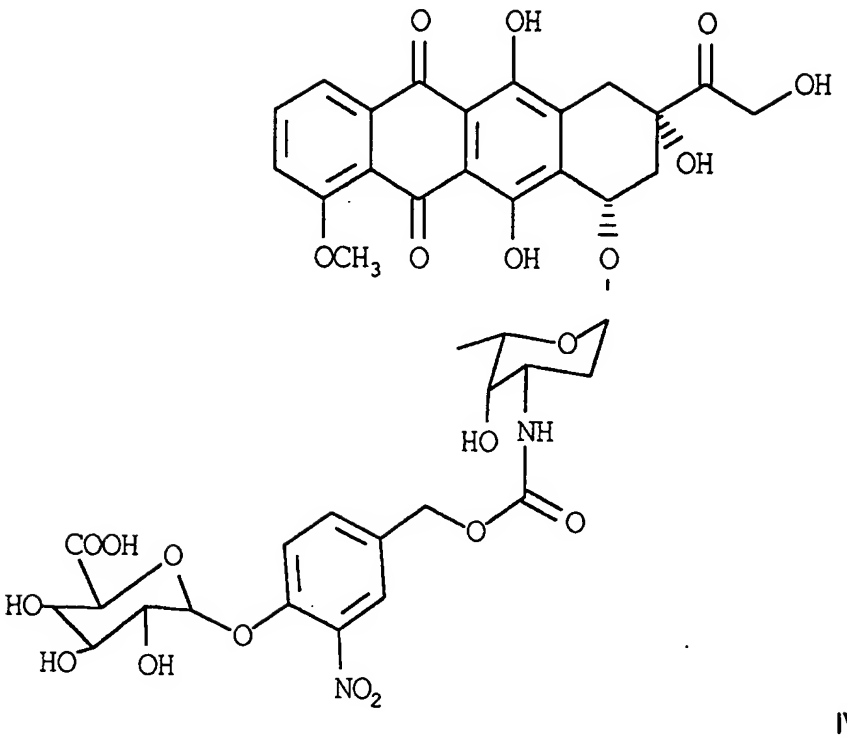
Example 11:



Example 12:



The pro-prodrugs defined in example 1 - 12 show an increased hydrophilicity compared to the parental prodrug of formula IV



IV

as shown by a reduced octanol water coefficient. This increased hydrophilicity leads to more favourable pharmacokinetics combined with a reduced toxicity and a more efficient site specific activation. This favourable observation is mediated by the action of at least two enzymes. The plasmatic esterases first convert the pro-prodrugs into prodrugs. These prodrugs are site specifically activated to toxic drugs by the action of a targeted glucuronidase and/or by lysosomal glucuronidases liberated in inflamed sites or in necrotic tumor tissues. The above mentioned two step activation mechanism results in a superior drug concentration at the target site compared to the drug concentrations obtained, if the MTD of the drug or the MTD of the parental prodrug is applied. As a consequence therapeutic efficacy is increased.
The use of this new site specific drug delivery systems thus results in a significant increase of the therapeutic window, which is defined as the ratio between the maximally tolerable dose and the minimal effective dose. Further improvements are achievable by encapsulation of these pro-prodrugs in stealth liposomes.

Example 13:

Pro-produgs (example 1) can be encapsulated according to D. Papahadjopoulos et al. (PNAS, USA 88:11460-11464, 1991) into stealth liposomes. After i.v. injection into CD1 nu/nu mice the plasma clearance of the pro-prodrug encapsulated into stealth liposomes should be prolonged from ≈ 20 min for the free pro-prodrug to ≈ 40 hrs for the encapsulated pro-produg. The significant t1/2β prolongation should lead to improved pharmacological efficacy.

Claims

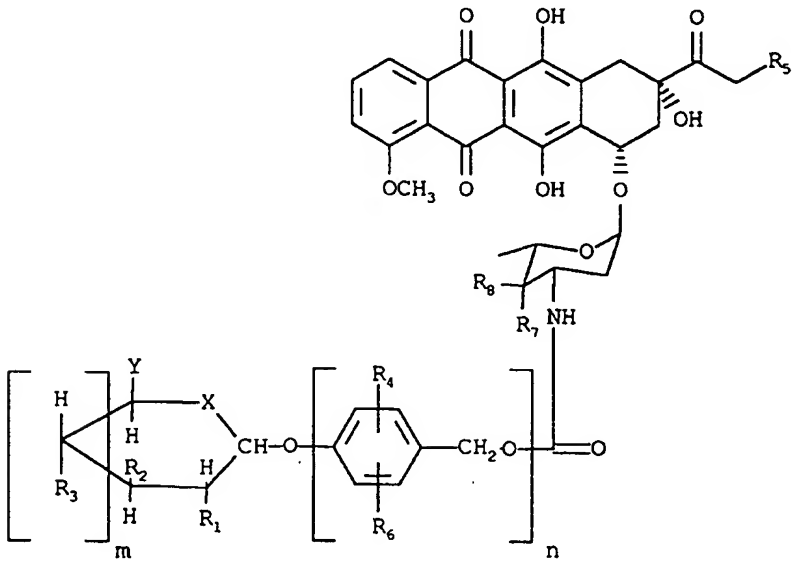
1. Compound according to formula 1,
   $(prodrug)_x$-S
   wherein
   S means a substrate moiety to which one or more (x = 1 or an integer) prodrug moieties can be bound and wherein the prodrug-S bond or bonds can be cleaved by one enzyme and where for optimal cleavage S can be linked to the prodrug moiety by a spacer which can be self-immolative
   wherein

prodrug means a moiety which is cleaved by another enzyme to generate a pharmacologically active substance.
wherein
the activating enzymes are at least two different human enzymes one of them located accessible only at the target site, the other one located systemically or in/at a different compartement/site of the body.

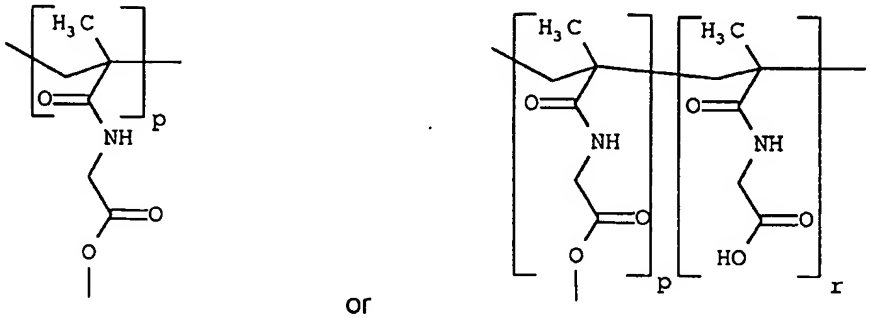
2. Compound according to claim 1, wherein the activating enzymes are hydrolases.

3. Compound according to claim 2, wherein the activating enzymes are esterases, preferentially phosphatases, combined with glycosidases, preferentially β-glucuronidases.

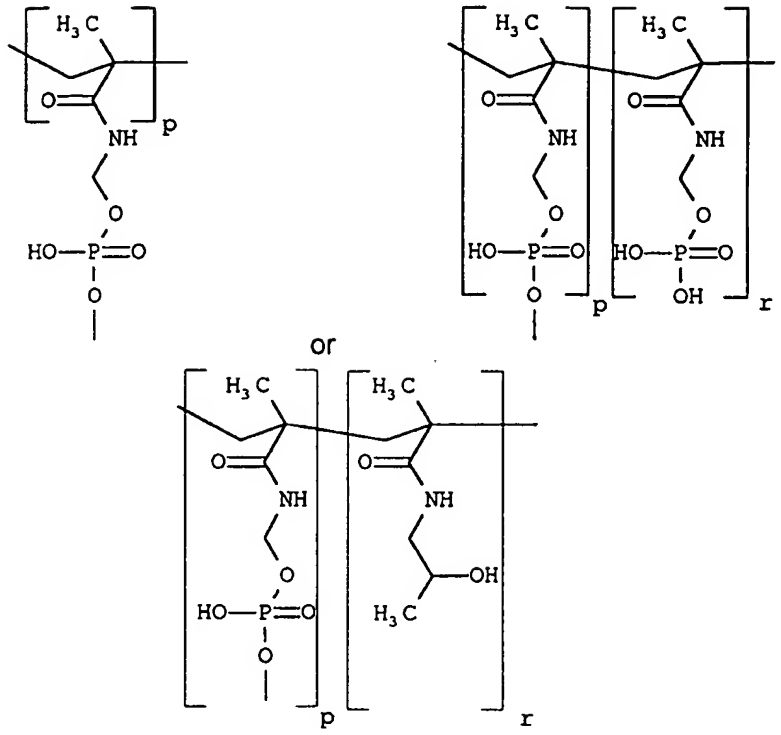
4. Compounds according to claim 1 with the general structure:



wherein
one of $R_{1,2,3,4,5,7,8}$ may be mono- or oligophosphate, sulfate, an acyl moiety such as $-OCO(CH_2)_o-COOH$ where o is 0 to 100, preferentially 0 to 30, a polymeric residue



where p and rare 0 to 100, preferentially 1-30,
a polyglutamat where p is 0 to 100, preferentially 1 to 30,
a phosphodiester



where p and r are 0 to 100, preferentially 1-30
or a phosphodiester substituted with polyethylene glycol or with polysialic acid
and the other R's are independently of each other H or OH,
wherein R_6 may be an electron withdrawing residue, preferentially a nitro-, fluoro- or chloro-residue,
wherein n is 0 or 1,
wherein X is O, NH or S,
wherein Y may be COOH, PO_3H_2, $-CH_2-COOH$, $-CH_2-PO_3H_2$, CHOH-COOH or CHOH-PO_3H_2,
wherein m is 0 or 1.

5. A pharmaceutical containing a compound as described in claims 1 to 4.

6. A pharmaceutical containing a compound as described in claims 1 to 4 in combination with pretargeted enzymes.

7. A pharmaceutical containing a compound as described in claims 1 to 4 encapsulated in liposomes.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 94 11 4195

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	EP-A-0 441 218 (BEHRINGWERKE AG.) * claims * ---	1-7	A61K47/48
D,Y	WO-A-92 19639 (LABORATOIRES HOECHST) * claims * ---	1-7	
X,Y	JOURNAL OF CONTROLLED RELEASE, vol.18, 1992, AMSTERDAM NL pages 123 - 132 V. SUBR 'POLYMERS CONTAINING ENZYMATICALLY DEGRADABLE BONDS, XII. EFFECT OF SPACER STRUCTURE ON THE RATE OF RELEASE OF DAUNOMYCIN AND ADRIAMYCIN FROM [N-(2-HAYDROXYPROPYL)-METHACRYLAMIDE] COPOLYMER DRUG CARRIERS IN VITRO AND ANTITUMOUR ACTIVITY MEASURED IN VIVO.' * figure 1 * --- -/--	1-7	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

A61K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	8 February 1995	Berte, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 94 11 4195

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X,Y	MAKROMOLEKULARE CHEMIE, vol.193, 1992, BASEL CH pages 1881 - 1887 YUICHI OHYA ET AL. 'SYNTHESIS AND CYTOTOXIC ACTIVITY OF DOXORUBICIN BOUND TO POLY(ALPHA-MALIC ACID) VIA ESTER OR AMIDE BONDS.' * tables 1,2 * ---	1-7	
X,Y	FARMACO, vol.48, no.7, 1993, PAVIA IT pages 919 - 932 P. CALICETI 'PREPARATION AND PROPERTIES OF MONOMETHOXYPEG-DOXORUBICIN CONJUGATES LINKED BY AN AMINO ACID OR A PEPTIDE AS SPACER.' * figure 1 * ---	1-7	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
X,Y	JOURNAL OF CONTROLLED RELEASE, vol.19, 1992, AMSTERDAM NL pages 331 - 346 R. DUNCAN ET AL. 'PRECLINICAL EVALUATION OF POLYMER-BOUND DOXORUBICIN.' * figure 1 * ---	1-7	
Y	GB-A-1 541 436 (SEARLE & CO) * claims * ---	1-7	
Y	WO-A-81 01145 (UNIVERSITY OF ILLINOIS FOUNDATION) -----	1-7	

INCOMPLETE SEARCH

Claims searched incompletely: 1-7

In view of the large number of compounds, which are designed by the general formulas of claim 1 and also in view of the definition of products by means of their biological, chemical and/or pharmacological properties, the search has to be restricted for economic reasons.
The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims or examples
(see guidelines, Part B, Chapter III, paragraph 3.6).